Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Jarr K-U, Nakamoto R, Doan BH, et al. Effect of CD47 blockade on vascular inflammation. N Engl J Med 2021;384:382-3. DOI: 10.1056/NEJMc2029834

Table of Contents **Page**

Methods

Study population and design

All participants enrolled at Stanford University from November 2016 through January 2020 in the ongoing Phase 1b/2 trial of magrolimab in combination with rituximab in relapsed/refractory B-cell non-Hodgkin's lymphoma (NCT02953509) were identified for inclusion in this retrospective analysis (total of 13 participants). These patients had refractory or relapsed B-cell lymphoma after two or more prior systemic therapies and the majority had become refractory to rituximab alone or in combination with chemotherapy prior to enrollment[1]. The protocol was reviewed and approved by the institutional review board at Stanford University (IRB# 55497). Participants were treated with magrolimab in combination with background rituximab therapy. Rituximab was administered intravenously at a dose of 375 mg per square meter of body surface area, weekly in cycle 1 starting in week 2, and then monthly in cycles 2 through 6[1]. Magrolimab was administered intravenously with a priming dose of 1 mg per kilogram of body weight, followed by weekly doses of 10 to 30 mg per kilogram in the first month, and weekly or Q2 weekly dosing in month 2 and beyond. In addition, a 50% dose escalation (to 45 mg per kilogram) was pre-specified in the event that lower doses were deemed safe[1]. Among the participants included in this retrospective study, only the doses of 20, 30, or 45 mg per kilogram were used. Baseline and follow-up ^{18}F-FDG-PET/CT scans were available for all study patients and were reviewed by 2 Nuclear Medicine physicians blinded to other examinations but aware of the protocol. Of note, all scans were de-identified and presented in a random order. Four scans were deemed uninterpretable for vascular uptake analysis due to extensive cervical lymphadenopathy

in the carotid sheath overlying the carotid artery and these patients were excluded. Fasting serum glucose was assessed at the time of the baseline and follow-up ^{18}F-FDG-PET/CT. Blood pressure values were taken within 7 days of the baseline and follow-up PET/CT scans.

^{18}F-FDG-PET/CT scans and analysis

All patients underwent ^{18}F-FDG-PET/CT before and at regular intervals after the administration of magrolimab. Baseline ^{18}F-FDG-PET/CT scans were obtained 12 days (mean ± SD: 12.1 ± 9.8) before therapy initiation. The first follow-up PET scans were performed 63 days (mean ± SD: 62.6 ± 33.5) after therapy initiation. Patients fasted for a minimum of 6 hours before intravenous ^{18}F-FDG administration. The time from injection to the start of the PET/CT scans was 72 minutes (mean ± SD: 71.7 ± 19.6). The baseline and first restaging PET/CT images were obtained in 3D mode from the vertex to the toes. The activity of ^{18}F-FDG administered ranged from 7.9 to 11.3 mCi (mean ± SD: 9.8 ± 1.1). PET/CT scans were acquired following procedure standards on Discovery 690, 710, or MI scanners (GE Healthcare, Waukesha, WI) in use at our institution. Both pre- and post-treatment scans were done using the same scanner. Of note, ^{18}F-FDG-PET/CT imaging has favorable interscan variability and low inter- and intra-observer variability[2], qualifying this modality for serial assessment and response to therapy[3,4]. Images were de-identified, presented in a random order, and analyzed using MIM Vista version 6.9.2 (MIM Software Inc., Cleveland, OH). The analyses were performed as previously described[4], and vascular uptake was quantified in the carotid arteries to avoid confounding by signal present in the mediastinal lymph nodes. Briefly,

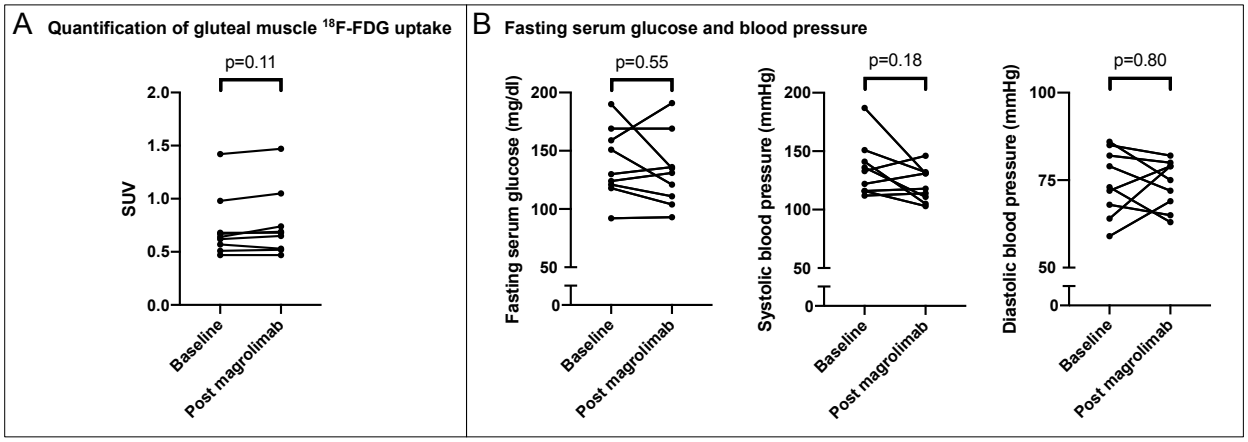
the carotid artery bifurcations on both sides were identified and arterial FDG uptake was measured starting 2 cm below the carotid artery bifurcation and continuing superiorly to 2 cm into the internal carotid artery. Measurements were made in the axial plane and maximum standardized uptake values (SUV) were obtained. The maximum target-to-background ratio (TBR) was calculated (ratio of the maximum SUV of the artery compared to background activity in the ipsilateral internal jugular vein). Next, the carotid artery with the highest FDG uptake was identified as the index vessel. The most diseased segment represented the arterial segment with the highest ^{18}F-FDG uptake at baseline scan. This was calculated as an average maximum TBR derived from four contiguous axial segments. Additionally, CT data were used to analyze the coronary artery calcium score as well as the carotid artery calcium score (index vessel) using Horos software (Horos Project; https://www.horosproject.org). The degree of coronary artery calcification was based on cutpoints (Score 0: absent; Score 1-100: mild; Score 101-400: moderate; Score >400: severe) commonly seen in the literature[5]. Additionally, to assess the physiological ^{18}F-FDG uptake in non-vascular tissue in these participants, we evaluated the mean SUV of the right gluteal muscle at baseline and post magrolimab using a spherical 2 cm volume of interest. No modifications or digital adjustments were applied to the representative PET/CT images in the Figure.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad, Inc., San Diego, CA). Data are presented as mean ± standard deviation (SD). Owing to the small sample size, PET/CT data were analyzed using a Wilcoxon matched-pairs signed rank

test (two-tailed) and no adjustment for multiplicity was made. A p value of 0.05 or less was considered to denote significance.

Supplementary Figure S1. Physiological ^{18}F-FDG uptake and traditional cardiovascular risk factors at baseline and post magrolimab.



Panel A shows the physiological ^{18}F-FDG uptake in the gluteal muscle (mean SUV). Panel B shows the available traditional cardiovascular risk factors fasting serum glucose and blood pressure. No effect was observed on these parameters after magrolimab therapy. Data from each patient before and after magrolimab treatment were compared and analyzed using a Wilcoxon matched-pairs signed rank test (two-tailed).

Supplementary Table S1. Baseline characteristics of the 9 patients included in the retrospective analysis.*

Characteristic	All patients (n=9)
Mean age (SD) – yr	71.0 (7.3)
Sex – no. (%)	
Male	7 (77.8)
Female	2 (22.2)
Mean body mass index (SD)†	30.2 (7.1)
Race – no. (%)‡	
White	7 (77.8)
Other	2 (22.2)
Risk factors and coexisting conditions – no. (%)	
Hypertension	8 (88.9)
Hyperlipidemia	5 (55.6)
Diabetes mellitus	4 (44.4)
Insulin therapy	3 (33.3)
Current smoker	0 (0)
Atherosclerotic disease§	6 (66.7)
Prior myocardial infarction	2 (22.2)
Medications – no. (%)	
Statin	4 (44.4)
Previous rituximab therapy (alone or in combination) – no. (%)	9 (100)

Mean time from magrolimab initiation to PET/CT scan (SD) – days	62.6 (33.5)
Coronary artery calcification score	
Mean Agatston score (SD)	324 (566)
Score: 0 – no. (%)	2 (22.2)
Score: 1-100 – no. (%)	3 (33.3)
Score: 101-400 – no. (%)	2 (22.2)
Score: >400 – no. (%)	2 (22.2)
Mean volume score (SD)	533 (818)
Mean mass score (SD)	449 (775)
Carotid artery calcification score	
Mean Agatston score (SD)	146 (185)
Mean volume score (SD)	287 (320)
Mean mass score (SD)	241 (280)

* Percentages may not total 100 because of rounding.

†The body mass index is the weight in kilograms divided by the square of the height in meters.

‡ Race was reported by the patient.

§ Atherosclerotic disease includes coronary artery disease, carotid artery disease, and atherosclerotic aortic disease.

Supplementary Table S2. Quantification of vascular ^{18}F-FDG uptake for each of the participants included in the retrospective analysis.

Participant	Baseline (n=9)	Post magrolimab (n=9)
Patient no. 1		
Maximum SUV	2.53	2.49
Maximum TBR	1.22	1.37
Patient no. 2		
Maximum SUV	1.71	1.53
Maximum TBR	1.44	1.34
Patient no. 3		
Maximum SUV	2.41	2.02
Maximum TBR	1.56	1.37
Patient no. 4		
Maximum SUV	3.74	2.34
Maximum TBR	1.46	1.23
Patient no. 5		
Maximum SUV	2.97	2.58
Maximum TBR	1.56	1.22
Patient no. 6		
Maximum SUV	2.49	1.51
Maximum TBR	1.47	1.14
Patient no. 7		
Maximum SUV	2.83	1.20

Maximum TBR	1.84	1.41
Patient no. 8		
Maximum SUV	3.23	2.37
Maximum TBR	1.55	1.10
Patient no. 9		
Maximum SUV	2.23	2.50
Maximum TBR	1.97	1.34

Supplementary Table S3. Variables of the patients at baseline and post magrolimab.

Variable	Baseline (n=9)	Post magrolimab (n=9)	P value*
Mean fasting serum glucose (SD) – mg/dl	139 (30)	132 (31)	0.55
Mean blood pressure (SD) – mmHg			
Systolic	135 (24)	121 (15)	0.18
Diastolic	74 (9)	74 (7)	0.80

* Data were analyzed by Wilcoxon matched-pairs signed rank test (two-tailed).

References

1. Advani R, Flinn I, Popplewell L, et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. N Engl J Med 2018;379:1711-21.

2. Rudd JH, Myers KS, Bansilal S, et al. (18)Fluorodeoxyglucose positron emission tomography imaging of atherosclerotic plaque inflammation is highly reproducible: implications for atherosclerosis therapy trials. J Am Coll Cardiol 2007;50:892-6.

3. Tahara N, Kai H, Ishibashi M, et al. Simvastatin attenuates plaque inflammation: evaluation by fluorodeoxyglucose positron emission tomography. J Am Coll Cardiol 2006;48:1825-31.

4. Tawakol A, Fayad ZA, Mogg R, et al. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. J Am Coll Cardiol 2013;62:909-17.

5. Budoff MJ, Nasir K, McClelland RL, et al. Coronary calcium predicts events better with absolute calcium scores than age-sex-race/ethnicity percentiles: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol 2009;53:345-52.